

AMENDMENTS TO THE SPECIFICATION

Kindly amend the title of the application as follows.

~~METHOD OF~~ METHODS FOR PRODUCING MINUS STRAND MINUS-STRAND RNA VIRUS VECTOR WITH THE USE OF VIRAL VECTORS USING HYBRID PROMOTER CONTAINING COMPRISING CYTOMEGALOVIRUS ENHANCER AND AVIAN CHICKEN β -ACTIN PROMOTER

Kindly insert the following heading and paragraph at page 1, line 6 of the English language specification.

Cross-Reference to Related Applications

This application is the U.S. National Stage of International Application No. PCT/JP2005/000705, filed January 20, 2005, which, in turn, claims the benefit of Japanese Patent Application No. 2004-014653, filed January 22, 2004.

Kindly amend the paragraph starting at page 8, line 12 of the English language specification as follows.

The chicken β -actin promoter includes a DNA fragment with promoter activity that comprises a transcription initiation site for the genomic DNA of the chicken β -actin gene. The nucleotide sequence of the chicken β -actin gene promoter has been reported by, for example, T. A. Kost et al. (Nucl. Acids Res. 11, ~~8287-8286~~ 8287-8301, 1983).

The chicken β -actin gene promoter is a gene fragment which has relatively a high G (guanine) and C (cytosine) content and contains sequences characteristic of promoters such as the TATA box (Ann. Rev. Biochem. 50, 349-383, 1981) and CCAAT box (Nucl. Acids Res. 8, 127-142, 1980). In the chicken β -actin promoter, the region from G (guanine) at position -909 to G (guanine) at position -7 upstream of the translation initiation codon (ATG) of the original β -actin structural gene is considered as an intron. Since this intron has transcription-promoting activity, it is preferable to use a genomic DNA fragment comprising at least a portion of this intron. Specifically, examples of this kind of chicken β -actin promoter include, for example, DNA comprising the nucleotide sequence of SEQ ID NO: 2. For the intron acceptor sequence, an intron acceptor sequence from a different gene is preferably used. For example, a splicing acceptor sequence of rabbit β -globin may be used. Specifically, the acceptor site of the second intron, which is located immediately before the initiation codon of rabbit β -globin, can be used. More specifically, such acceptor sequences include, for example, DNA comprising the nucleotide sequence of SEQ ID NO: 3. A CA promoter of the present invention is preferably a DNA in which a chicken β -actin promoter comprising a portion of the intron is linked downstream of a CMV IE enhancer sequence and a desired intron acceptor sequence is added downstream thereof. An example is shown in SEQ ID NO: 4. To express a protein, the last ATG in this sequence is used as the start codon and the coding sequence for the protein of interest may be linked thereto. To transcribe a minus-strand

RNA viral genome, DNA encoding the minus-strand RNA viral genome or the complementary strand thereof (either a plus or minus strand) is linked downstream of the intron acceptor sequence described above. However, as described below, it is preferable to insert a DNA encoding a self-cleaving ribozyme between the intron acceptor sequence and the DNA encoding a minus-strand RNA viral genome.

Kindly amend the paragraph starting at page 16, line 10 of the English language specification as follows.

Herein, a minus-strand RNA virus refers to viruses that contain a minus strand (an antisense strand complementary to a sense strand encoding viral proteins) RNA as the genome. The minus-strand RNA is also referred to as negative strand RNA. The minus-strand RNA virus used in the present invention particularly includes single-stranded minus-strand RNA viruses (also referred to as non-segmented minus-strand RNA viruses). The “single-strand negative strand RNA virus” refers to viruses having a single-stranded negative strand [*i.e.*, a minus strand] RNA as the genome. Such viruses include viruses belonging to Paramyxoviridae (including the genera *Paramyxovirus*, *Morbillivirus*, *Rubulavirus*, and *Pneumovirus*), Rhabdoviridae (including the genera *Vesiculovirus*, *Lyssavirus*, and *Ephemerovirus*), Filoviridae, ~~Orthomyxoviridae~~, (including Influenza viruses A, B, and C, and Thogoto-like viruses), Bunyaviridae (including the genera *Bunyavirus*, *Hantavirus*, *Nairovirus*, and *Phlebovirus*),

~~Arenaviridae~~, and the like.

Kindly amend the paragraph starting at page 29, line 14 in the English language specification as follows.

In a method of transcribing the minus-strand RNA virus genome by a bacteriophage RNA polymerase, it is possible to use 0.1 to 2 µg (more preferably 0.5 µg) of an NP-expressing plasmid, 0.1 to 2 µg (more preferably 0.5 µg) of a P-expressing plasmid, 0.5 to 4.5 µg (more preferably 2.0 µg) of an L-expressing plasmid, 0.1 to 5 µg (more preferably 0.5 µg) of an F-expressing plasmid, a T7 RNA polymerase-expressing plasmid (for example, 0.5µg), and 0.5 to 5 µg (more preferably 5 µg) of a viral genome RNA-encoding plasmid (plus or minus strand). For producing SeV, for example, the plasmids described in the Examples can be used in the following amounts:

pCAGGS-NP	0.1 to 2 µg (more preferably, 0.5 µg)
pCAGGS-P	0.1 to 2 µg (more preferably, 0.5 µg)
pCAGGS-L(TDK)	0.5 to 4.5 µg (more preferably, 2.0 µg)
pCAGGS-F5R	0.1 to 5 µg (more preferably, 0.5 µg)
<u>pCAGGS-T7</u>	<u>for example, 0.5 µg</u>
pCAGGS-SeV <u>pSeV(TDK)18+GFP</u>	0.5 to 5 µg (more preferably, 5 µg)
(pCAGGS-SeV/ΔF-GFP) (pSeV/ΔF-GFP)	

Kindly insert the sequence listing enclosed herewith at the end of the specification.